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Dated 18 September 2003

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30AUG02 E744726-11 D02819
P01/7700 0.00-0220156.4

Request for grant of a patent

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1. Your reference

HCM/C1384.00/M

2. Patent application number
(The Patent Office will fill in this part)

0220156.4

30 AUG 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Medical Research Council
20 Park Crescent
London
W1N 4AL

Patents ADP number *(if you know it)*

5840624001

If the applicant is a corporate body, give the country/state of its incorporation

England

4. Title of the invention

Optical Projection Tomography

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Keith W Nash & Co
90-92 Regent Street
Cambridge
CB2 1DP

Patents ADP number *(if you know it)*

1206001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	9 CF
Claim(s)	2
Abstract	1
Drawing(s)	3 f 3

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Keith W Nash & Co Date 29.08.2002

12. Name and daytime telephone number of person to contact in the United Kingdom: Clare Matthews - 01223 355477

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Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.*

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TITLE: OPTICAL PROJECTION TOMOGRAPHY

Field of the Invention

This invention relates to optical projection tomography.

Background to the Invention

Optical projection tomography is a known technique for producing three-dimensional images of specimens. For example, in US Patent 5,680,484 a specimen is rotated to a number of indexed positions so that light directed at the specimen produces a series of images at successive rotational positions of the specimen. These images are fed into a radon transform which constructs a three-dimensional image of the specimen.. The invention aims to provide a different way of directing the light onto the specimen, with a view to reducing noise or interference in the series of images and providing improved depth of focus in the series of images.

Summary of the Invention

According to one aspect of the invention there is provided apparatus for obtaining an image of a specimen by optical projection tomography, the apparatus comprising light-scanning means and a rotary stage for rotating the specimen to indexed positions in each of which the specimen is in use subjected to a scanning movement of incident light by the scanning means.

The incident light may be scanned in a direction perpendicular to an optical axis defined by the light passing through the apparatus.

The light scanning means may form part of a confocal scanning microscope.

According to another aspect of the invention there is provided a method of obtaining an image of a specimen by optical projection tomograpy, the method comprising scanning the specimen with a light beam and detecting light emanating from the specimen to derive the image.

Preferably, the detector detects light which is unscattered.

The incident light is preferably scanned in a raster pattern, one complete scan being undertaken at each indexed position of the specimen.

Brief Description of the Drawings

The invention will now be described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 is a diagram of the apparatus,

Figures 2a and 2b show how the microscope optics of the apparatus can be arranged to have low numerical aperture or high numerical aperture,

Figure 3 shows known image-forming optics,

Figures 4 and 5 show the image-forming optics of an optical system of the inventive apparatus,

Figures 6a, 6b, 6c and 6d show representative light paths for the optical system of the inventive apparatus,

Figures 7a, 7b and 7c illustrate how different degrees of refraction affect operation of the optical system,

Figure 8 illustrates how refraction is measured using a one-dimensional array of detectors; and

Figures 9 to 12 illustrate, in three dimensions, the operation of the optical system.

Detailed Description of the Drawings

Referring to Figure 1, the apparatus comprises a light source 1 (in the form of a laser) which supplies light to a two-dimensional light scanning means 2, the scanning mechanism of which has a dual mirror system. Light with a scanning motion is fed through image-forming optics 3. A dichroic mirror 4 interposed between the light source 1 and the scanning means 2 directs returned light to a high speed light detector 5. The components 1 to 5 may be provided by a confocal light-scanning microscope.

Light from the optics 3 passes through a specimen 6 which is rotated within, and supported by, a rotary stage 7 which in structure corresponds to the rotary stage disclosed in the applicant's co-pending International Patent Application No. PCT/GB02/02373. The rotary stage 7 rotates the specimen 6 to successive indexed positions at each of which one complete scan of the excitation light is undertaken. After passing through the specimen 6, the light is processed by an optical system 8 which directs the light to a one-dimensional or two-dimensional array of high speed light detectors 9.

In fluorescence mode, light from the specimen 6 is returned through the optics 3 and the scanning means 2 and thence, via the mirror 4, to the high speed light detector 5. In this method of fluorescence imaging, the excitation light enters one side of the specimen and leaves the specimen from the same side thereof before being detected. It is in the

transmission mode, to be described, that the components shown to the right of the stage 7 in Figure 1 are used.

The microscope optics 3 may have a high numerical aperture (Figure 2a) or may be adapted to have a low numerical aperture (Figure 2b) which is useful for some specimens to be imaged.

Figure 3 illustrates a known image-forming system. The light from any point on the focal plane 12 (within the specimen) is collected and refracted by a lens 13 towards a single point in the image plane 14. There exists a symmetry such that any point on the image plane 14 maps to a point in the focal plane 12 and *vice versa*.

By contrast, the need for an *image-forming* optical arrangement is removed in the "non-focal" optics of Figures 4 and 5 which displays no such symmetry. The non-focal optical system 8 is represented by a convex lens 15. The light from a single point on the focal plane 12 is not focussed onto a single light detector. It is diverged such that only the light which continues to travel straight through the specimen 6 along the optical axis of the apparatus (i.e. the light 16 which is not scattered) reaches the single light detector 9a positioned on the optical axis. The purpose of the lens 15 in this case is very different from Figure 3. It can only function in a light-scanning situation. The narrow light beam is scanned (e.g. in a raster pattern) across the specimen through a multitude of different positions (five of which are illustrated as the black arrows in Figure 5). The purpose of the non-focal optical system 8 (i.e. the lens 15) is to direct only unscattered and unrefracted light onto the single light detector 9a, irrespective of the scanning position of the light beam. In specimens which cause significant scattering of light the system allows a higher signal-to-noise ratio to be obtained by limiting detection of scattering light. Alternatively, the specimens with low scattering but a non-uniform distribution of refractive index the system allows this non-uniform distribution to be calculated by measuring the degree of refraction experienced by each projection.

Figures 6a to 6d illustrate some representative light paths for rays (derived from a narrow laser beam) emitted from the specimen 6 while passing through the non-focal optical system.

In Figure 6a rays scattered from a point in the centre of the specimen 6 are diverged away from the light detector 9a. The proportion of scattered rays which are detected can be adjusted by changing the effective size of the detector. An adjustable iris allows this control (which is very similar to the pin-hole in a scanning confocal microscope). Alternatively, the position of the lens can be adjusted to cause more or less divergence of the scattered rays. In optical image-forming systems, an airy disc is the interference pattern produced by the light emitted from a single point within the specimen. Optical systems which produce larger airy discs have lower resolving power, as airy discs from neighbouring points within the specimen will overlap. The concept of the airy disc is not strictly relevant to a project-measuring system like this, however a similar concept does exist. In the case of the non-focal optics described here, light from each projection creates a very broad distribution of intensities (at the position of the detector) similar to a broad airy disc, which might suggest low resolving power. However, as only a single projection is measured at any one time even very broad distributions cannot interfere with each other.

In Figure 6b rays scattered from other points along the same line sampled in Figure 6a, are also diverged away from the light detector 9a.

In Figure 6c unscattered light from a different scanned position (black arrow) is emitted from the specimen 6 substantially parallel to the optical axis, and is therefore refracted towards the light detector 9a. As in Figures 6a and 6b, scattered light is directed away from the detector 9a

In Figure 6d unscattered rays from any scanned position are directed onto the light detector 9a. The arrows represent successive positions of the laser beam as it is scanned across the specimen 6 in a direction perpendicular to the optical axis.

All experiments done so far with optical projection tomography have had to assume that although some of the light is scattered, the refractive index of the specimen is uniform. Recent experiments have demonstrated that a number of important specimens (including medical imaging of biopsies) display non-uniform refractive indexes. This means that the current algorithms are not accurately imaging the specimen – distortions and artefacts are introduced. The apparatus described reduces this problem by measuring information not previously available – i.e. the angle at which a light beam exits from the specimen.

In the use of the present apparatus a clearing agent (such as BABB) is used such that the majority of the light is not scattered. It is however subject to a different form of disruption – refraction. In Figure 7, scattered light is indicated by broken lines, while the main path of light is shown in solid lens. In the first example of Figure 7a this path is not bent as it passes through the specimen 6 (it is only refracted on passing through the lens). The main path does pass through a region of the specimen with a higher refractive index than the rest (grey disc), however both the interfaces it encounters between regions of differing refractive index are perpendicular to the light path, so no refraction occurs.

In the second case of Figure 7b, the illumination beam is slightly higher and therefore the interfaces it encounters between the grey region and the white region of the specimen (different refractive indexes) are slightly displaced from perpendicular. This causes two slight refractions of the main path such that when the light emerges from the specimen it is no longer parallel to the optical axis and is directed slightly to the side of the original central light detector 9a. If auxiliary light detectors 9b are positioned on either side of the central detector 9a, these can measure the degree of refraction. Any projection will give a certain distribution of intensities along the array of light detectors, with the strongest values closer to the centre of the distribution. The system need only determine where the centre of this distribution is (usually the strongest intensity) to measure the angle at which the main light path emerged from the specimen. In the last case of Figure 7c, a different scanned position has caused greater refraction of the beam, which is reflected in a further shift along the array of detectors.

In Figure 8, an oblong region of the specimen 6 has a higher refractive index (grey shape) than the rest. Rays passing around the specimen are not refracted and so are directed to the central light detector 9a. Rays passing through the middle of the specimen (middle two rays in Figure 8) are refracted twice. The two interfaces which the light passes through (white-to-grey and then grey-to-white) are parallel with each other, and the light rays therefore exit the specimen at the same angle that they entered it. These rays are also directed onto the central detector 9a, as if they had not been refracted. Rays passing through other parts of the grey region are also refracted twice but not passing through parallel interfaces, so these rays are detected by the adjacent light detectors 9b.

The fact that some rays will be refracted and still exit the specimen 6 in the same orientation (although a different position) is not a problem. The example of Figure 8 shows only one of the many sets of projections taken through this section. Full imaging involves capturing such a data set for hundreds of orientations through the section, and the combination of all this data allows a full reconstruction of the distribution.

Figures 9 to 12 show three-dimensional views of the apparatus. In Figure 9, all un-refracted (and unscattered) rays through a two-dimensional section of the specimen are focused onto the central light detector of the array. The specimen 6 is rotated about a vertical axis between indexed positions in each of which a complete scan is undertaken.

Figure 10 shows the path of scattered or refracted light onto auxiliary light detectors.

Figure 11 illustrates that the lens (or optical system) allows the one-dimensional array of detectors 9 to capture data from a full two-dimensional raster-scan of the specimen. A row of scanned positions is always directed down or up to the row of detectors, irrespective of the vertical height of the scan.

A two-dimensional array of light detectors 9 may be used instead of a one-dimensional array, as shown in Figure 12. This would be able to measure light which is scattered above or below the plane occupied by the light rays shown in Figure 12.

The data derived from the detector array 9 optics is interpreted by an algorithm.

Many different algorithmic approaches already exist for performing back-projection calculations. One approach is to use a standard linear filtered back-projection algorithm (as in US Patent 5680484). Other approaches include iterative, maximum entropy and algebraic reconstruction technique. (R. Gordon et al., "Three-Dimensional Reconstruction form Projections: A Review of Algorithms".

The algorithm works as follows:

1. The data is used as if it were parallel (or fan-beam) data to perform back-projection. This produces a "fuzzy" estimation of the distribution of absorption or fluorescent characteristics of the specimen.

2. A first approximation of the distribution of refractive index is estimated. This can be done in a number of ways. One useful method is to assume that the absorption or fluorescent distribution will reflect the distribution of refractive index. Within each section a 2-D gradient vector is calculated for each voxel. An alternative is to start with a uniform or a random distribution.

3. The estimated refraction distribution is used to perform a forward-projection, i.e. a prediction of what the projection data should look like if the initial estimate of the refraction distribution was correct.

4. The predicted projections and the actual projections are compared.

5. The estimated refraction distribution is modified. The projections with a greater difference between predicted and actual, pin-point which regions of the distribution need more modification. For example, in the case of the grey shape shown in Figure 8, projections from the curved ends of the oblong will differ greatly from the

predictions due to the large amount of refraction. Voxels in the regions therefore have their predicted refraction indexes changed more than other regions.

6. The loop from 3 to 6 is repeated until no further improvements to the predicted projections can be made.

CLAIMS

1. Apparatus for obtaining an image of a specimen by optical projection tomography, the apparatus comprising light scanning means and a rotary stage for rotating the specimen to indexed positions in each of which the specimen is in use subjected to a scanning movement of incident light by the scanning means.

2. Apparatus according to claim 1, wherein the incident light is scanned in a direction perpendicular to an optical axis followed by the light passing through the apparatus.

3. Apparatus according to claim 1 or 2, wherein the incident light is scanned in a raster pattern, one complete scan being undertaken at each indexed position of the specimen.

4. Apparatus according to any of the preceding claims, wherein the light scanning means form part of a confocal scanning microscope.

5. A method of obtaining an image of a specimen by optical projection tomography, comprising scanning the specimen with a light beam and detecting light emanating from the specimen to derive the image.

6. A method according to claim 5, wherein the light passes through the specimen prior to being detected.

7. A method according to claim 5, wherein the light enters from one side of the specimen and leaves the specimen from the same side thereof.

8. A method according to any of claims 5 to 7, wherein the specimen is rotated to indexed positions and one complete scan is undertaken at each indexed position of the specimen.

9. A method according to any of claims 5 to 7, wherein the detector detects light which is unscattered.

10. A method according to any of claims 5 to 9, wherein the light is laser light.

ABSTRACT

TITLE: OPTICAL PROJECTION TOMOGRAPHY

Apparatus for obtaining an image of a specimen (6) by optical projection tomography comprises a light scanner, such as a light-scanning confocal microscope (1, 2, 3) for subjecting the specimen (6) to a scanning movement of incident light.

[Figure 1]

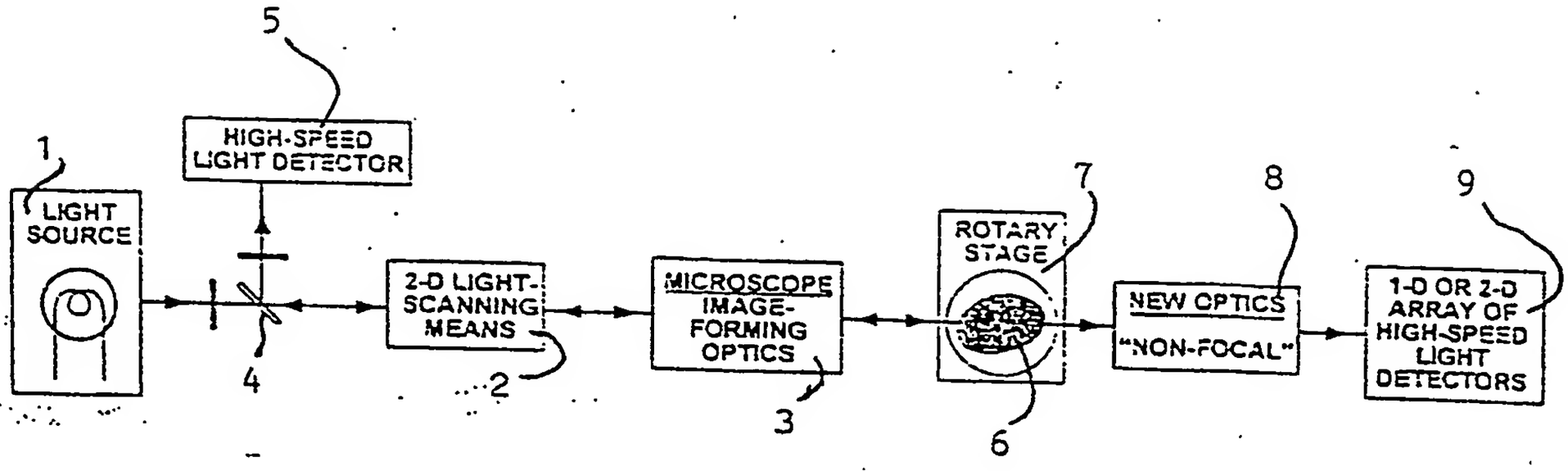


Figure 1

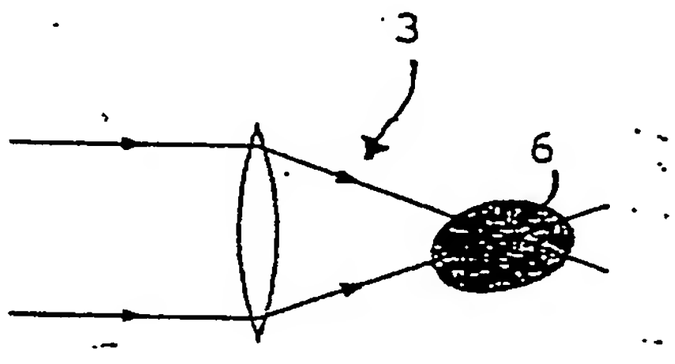


Figure 2a

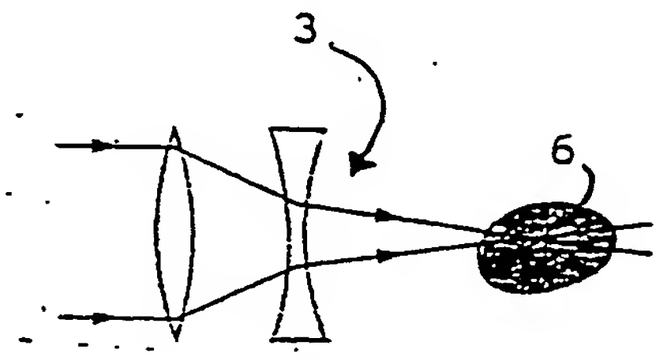


Figure 2b

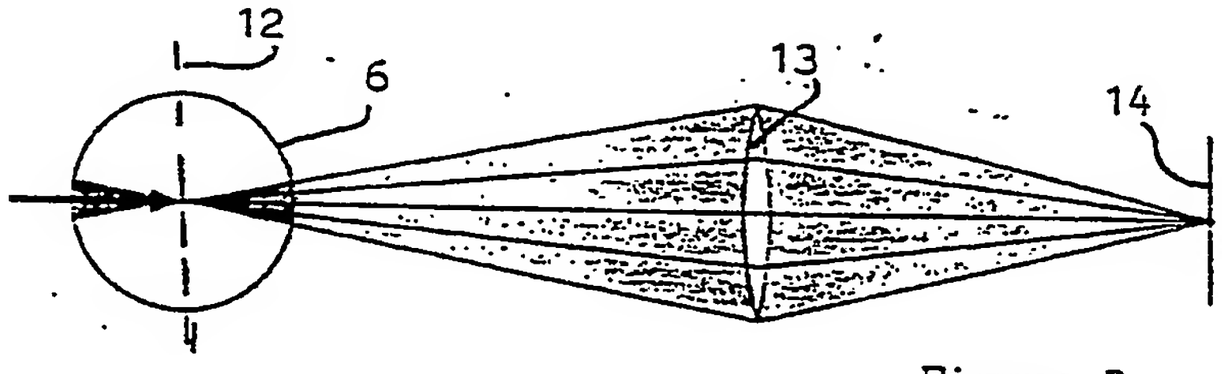


Figure 3

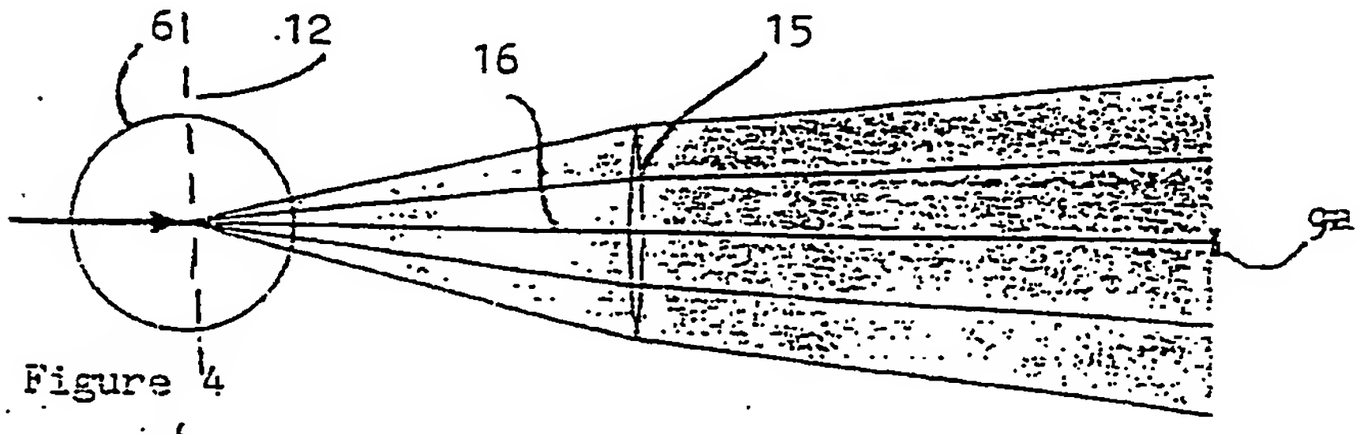


Figure 4

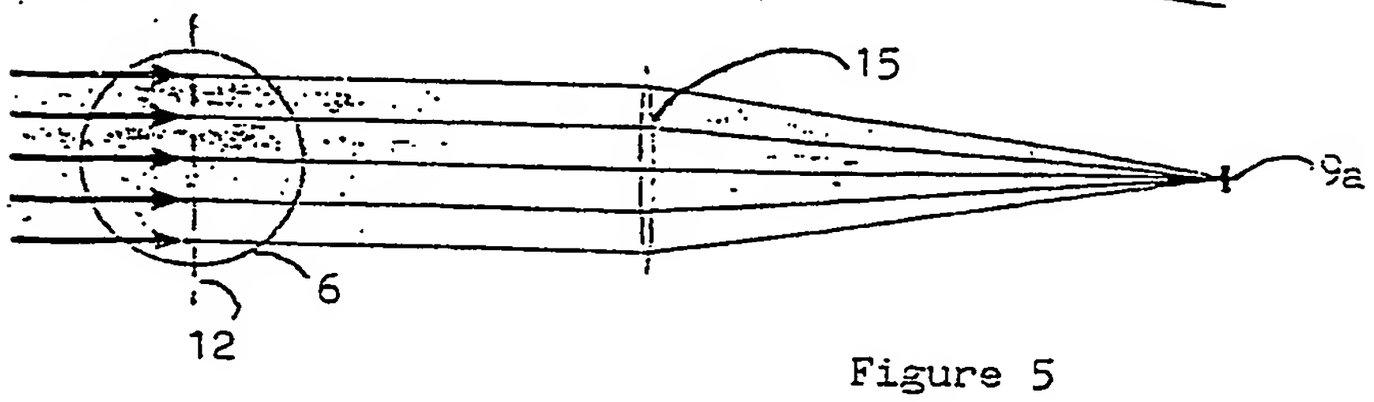


Figure 5

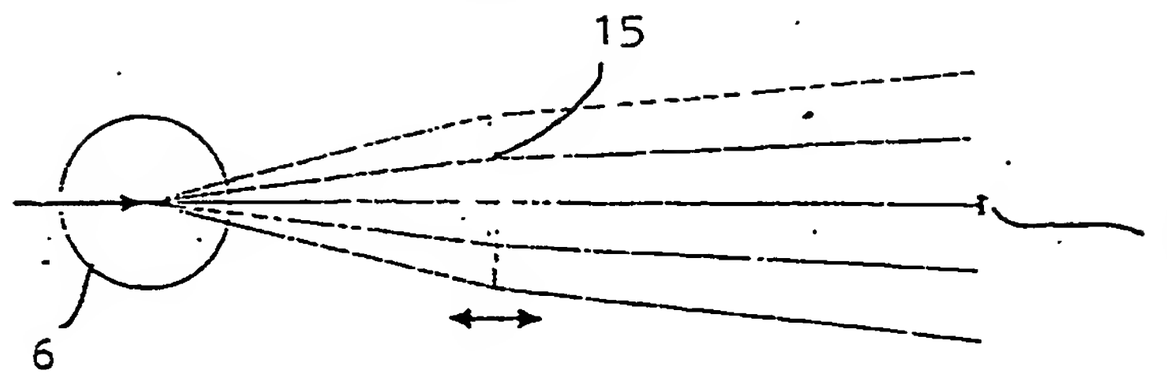


Figure 6a

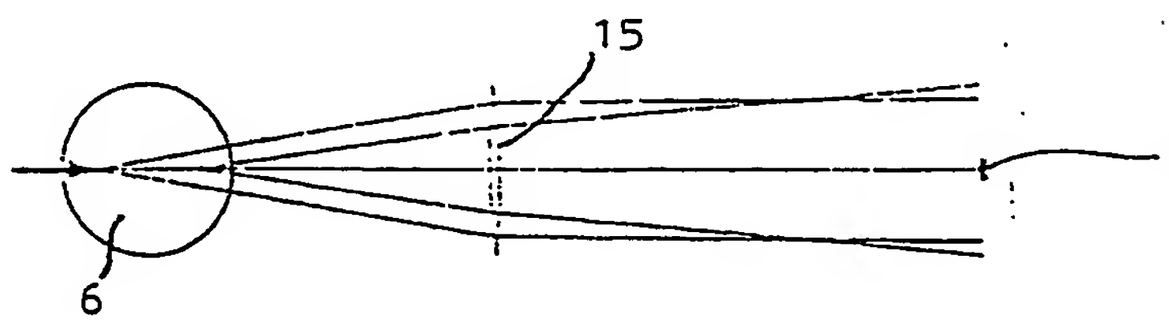


Figure 6b

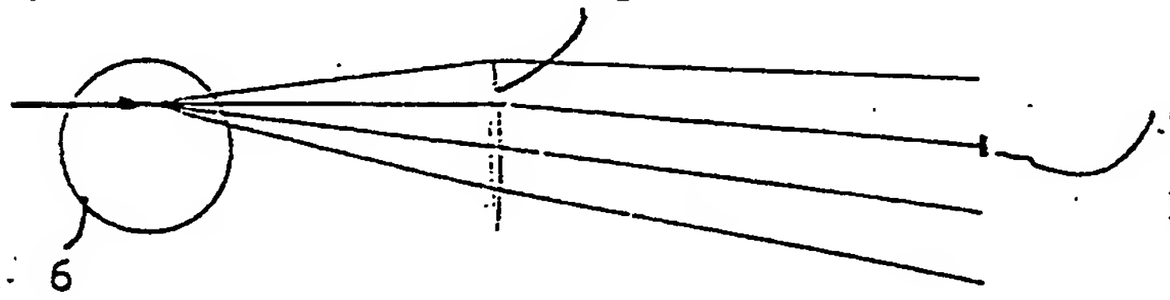


Figure 6c

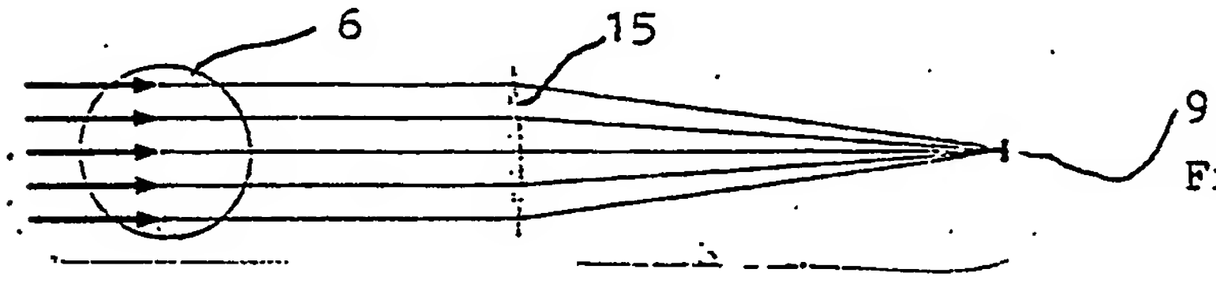


Figure 6d

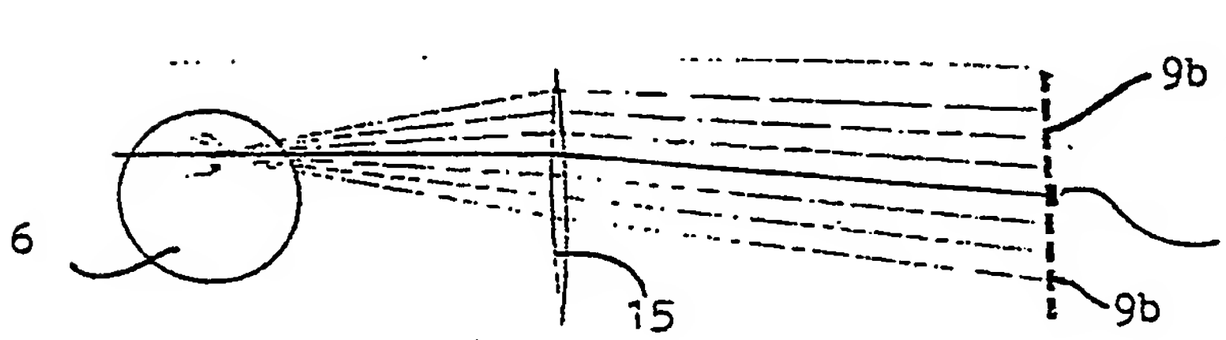


Figure 7a

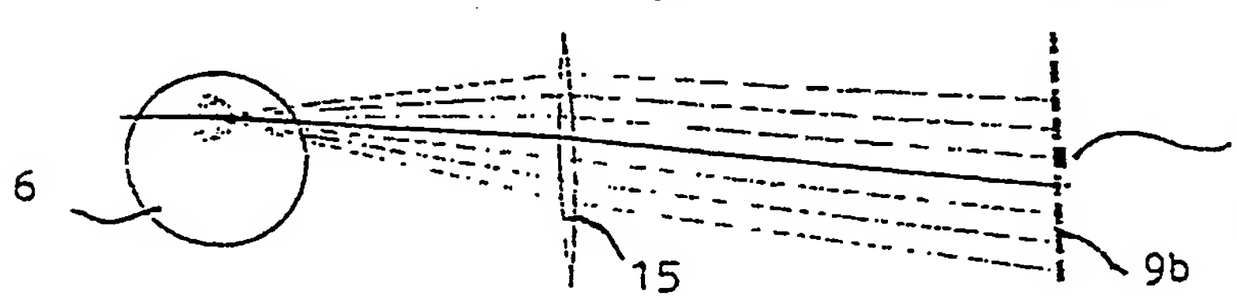


Figure 7b

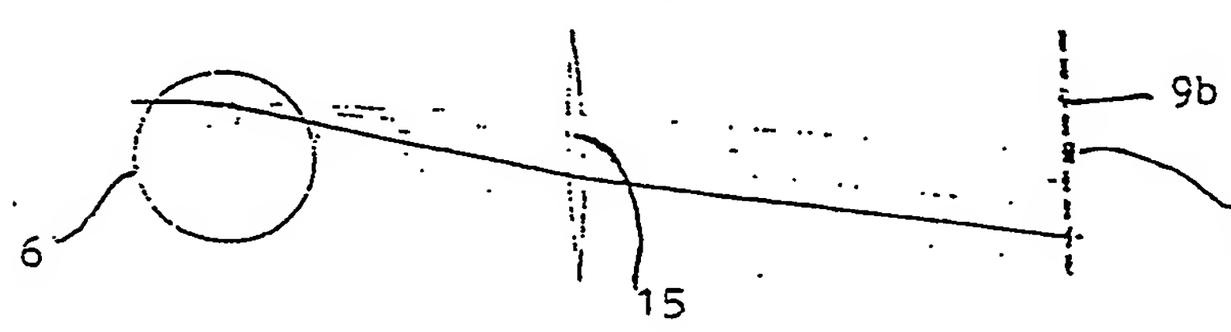


Figure 7c

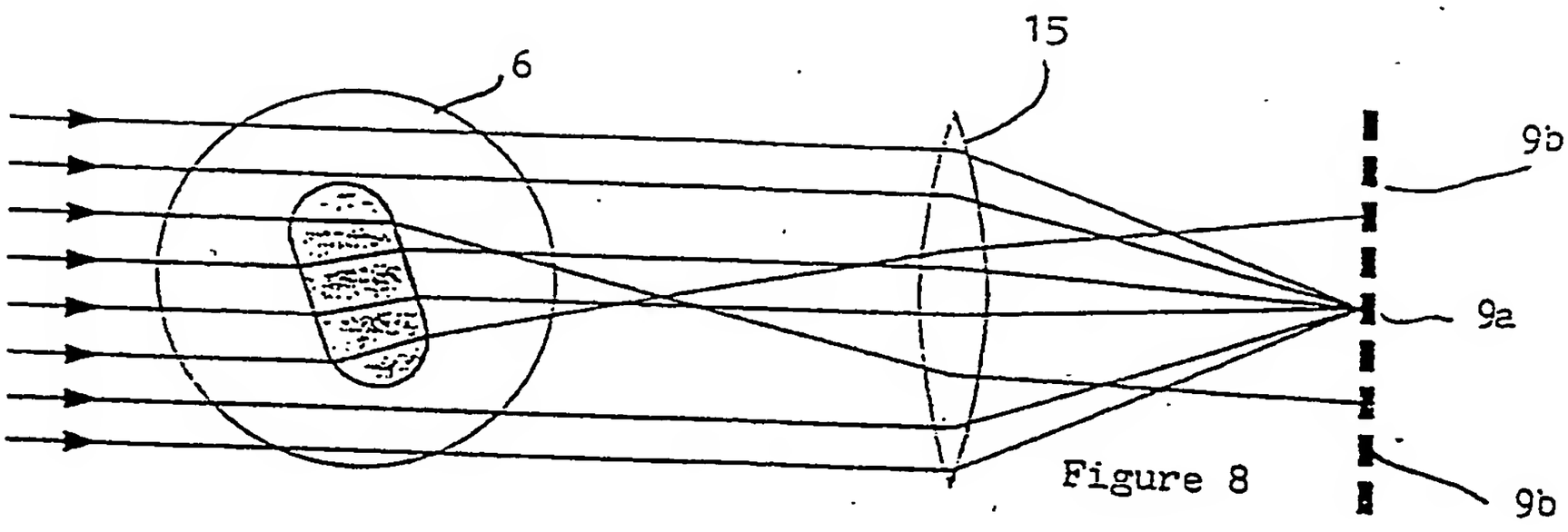


Figure 8

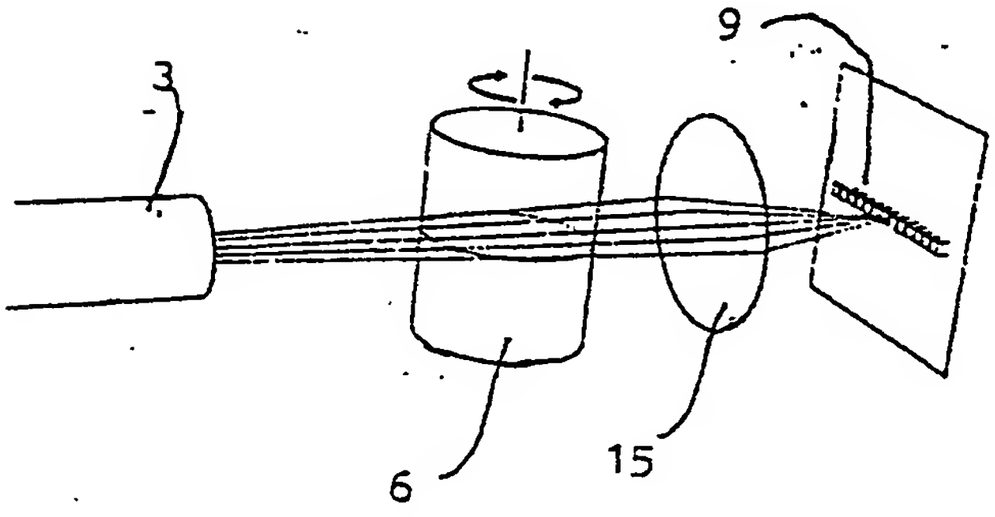
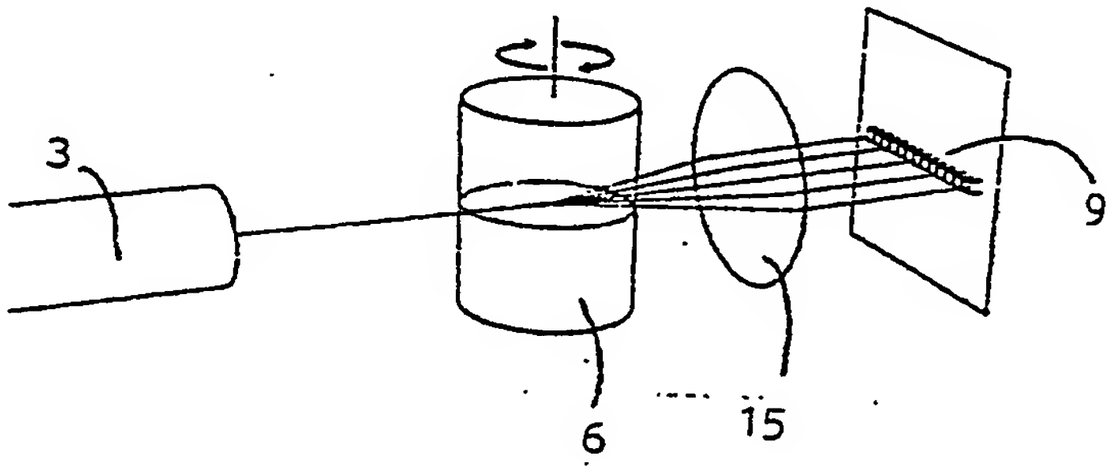


Figure 9

Figure 10



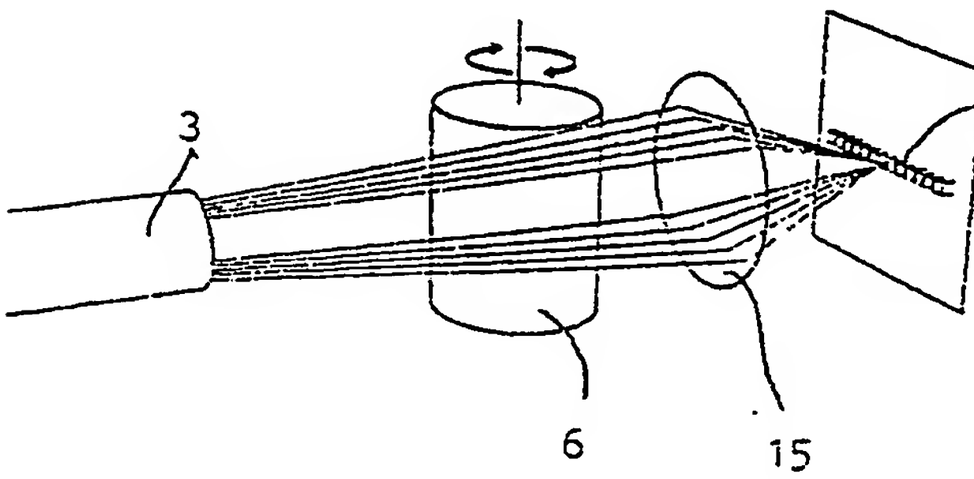
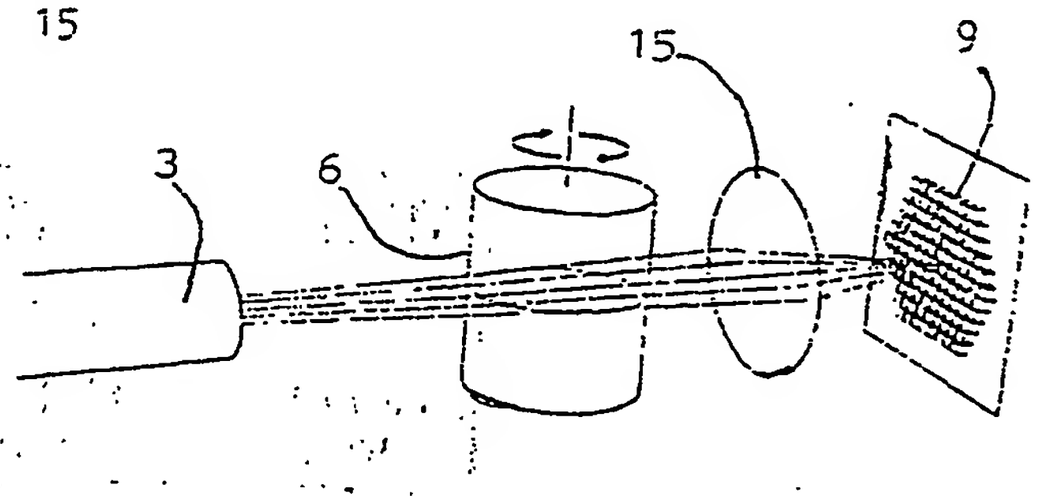


Figure 11

Figure 12



PCT Application

GB0303746